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SPECIFIC DEATH SITES IN A *DROSOPHILA* POPULATION CAGE

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I wish to report the observation that when population cages of *Drosophila melanogaster* are provided with empty vials, more than half of the flies die in these vials rather than elsewhere. This phenomenon has been followed continuously over a period of some $1\frac{1}{2}$ years, in a dozen cages, and under a variety of conditions. It bears on the nature of territoriality and on the control of population density.

Materials and Methods

Populations of *Drosophila melanogaster* were established in lucite population cages (boxes $135 \times 110 \times 450$ mm o.d., on 115 mm supports, screen-vented at each end, and fitted with 20 standard 25×95 mm glass culture vials in two rows). The cages were located on a counter in my laboratory at room temperature. Light was not controlled. All but two culture vials contained about 10 cc of standard culture medium and were replaced at staggered intervals, so that a complete age array of *Drosophila cultures* was present at all times. The remaining two vials were empty; these were placed in terminal positions and removed at certain intervals for counting. Before the empty "death" vials were removed for counting, they were tapped repeatedly to encourage flies to depart that might be casual transients. The remaining flies were etherized; those that recovered were designated "moribund," and those that did not were called "dead." Ideally, the ether would finish off no living flies, and this appears to have been essentially the case: most flies in the death vials were clearly dead. The moribund flies exhibited characteristic behavior.

Results

A log of the running observations over a 16-month period is presented in Figure 1. The data were collected, first from the death vials of 9, then from 4 cages, and they consist of two variables: number of dead flies per vial and number of moribund flies per vial at the time of counting. These data do not include flies dying or becoming moribund elsewhere in the cages.

Table I presents these and derivative data for certain time periods. At the outset, Standard Period 1, the two death vials in each cage were at the end facing into the room. In Figure 1, a sharp oscillation can be seen: the right hand vials were nearer the major source of light, a window, and they almost invariably contained more flies.

The cages were then reversed, so that the death vials were now against the wall, where it was darker. Now the number of flies dying in the death vials dropped sharply, although the number of moribund flies did not. The right-left difference disappeared, also. When the cages were turned around once more

TABLE I

Calculation of death rates

Sample period	Experimental time (days)	Dead (N)	Dead day/vial	Moribund (N)	Dead + Moribund	Vial days	Replicates (N)	Death rate**
Standard I	64–153	45,397	26.97	15,366	60,763	187	9	36.10
Reverse*	164–216	3,842	8.50	3,320	7,162	113	4	15.85
Standard II	223–263	8,872	25.49	3,149	12,021	87	4	34.54
Foil and Hood*	271–331	12,460	24.34	5,088	17,548	128	4	34.27

* See text and Figure 1.

** Dead and Moribund Flies divided by (Vial Days × Replicates).

(Standard Period II), the results returned to their original values, including the right-left difference (Figure 1). Next, the death vials were covered with foil (D) and then additionally the right hand sides of the cages were covered with foil (E). Finally (F), foil or mylar hoods were placed over each cage. Now the observations were similar to those seen under the initial conditions, except that the oscillations due to right-left differences were not seen. To interpret these results simply, it appears that light does attract the flies, even the moribund ones, but the influence of light is an epiphenomenon: in the near absence of light, the dying flies accumulate in the death vials to the same extent as in light.

It may be noted that the death vial floors occupy about 1.7% of the total floor area of the cage, so that the accumulation of moribund and dead flies in these vials is anything but random. Vials placed horizontally in a port at the end of the cages accumulated very few dead flies (1.6 per vial per day).

Table II illustrates the ratio between moribund flies per vial (at time of sampling) and dead and moribund flies per vial per day. In a steady state situation, the rate of arrival of moribund flies equals the death rate. The ratio of moribund flies present at any time to the death rate should equal the duration of the moribund period, and this works out to about 46 hours. In contrast, in a recent experiment, healthy flies lived a median time of 23 hours in empty vials under ambient conditions of temperature and humidity, and 34 hours if the cotton plug was moistened. Since the moribund flies entering the death vials are not even representative healthy flies, we might have expected them to last less than 24 hours. The fact that they appear to live on for almost 2 days suggests that some moribund flies do reenter the arena and eat once or occasionally more often.

The distinction between moribund flies and ordinary healthy flies is illustrated by the results of the following experiment. Over 400 moribund flies (see materials and methods for criterion) were collected and placed in ordinary food vials, at the low density of 20 per vial. The survivors in each vial were transferred to a new vial at roughly 5-day intervals. Median survival time was 26 days, and the survival curve was smooth, not stepped. Thus, the flies seem to have constituted a fairly continuously variable group, not a mixture of more than one distinct class. A comparable group of over 400 flies attracted from the main part of the cage by light and treated similarly showed a median survival time of 52 days. Again the curve was smooth and not stepped. Moreover, abundant

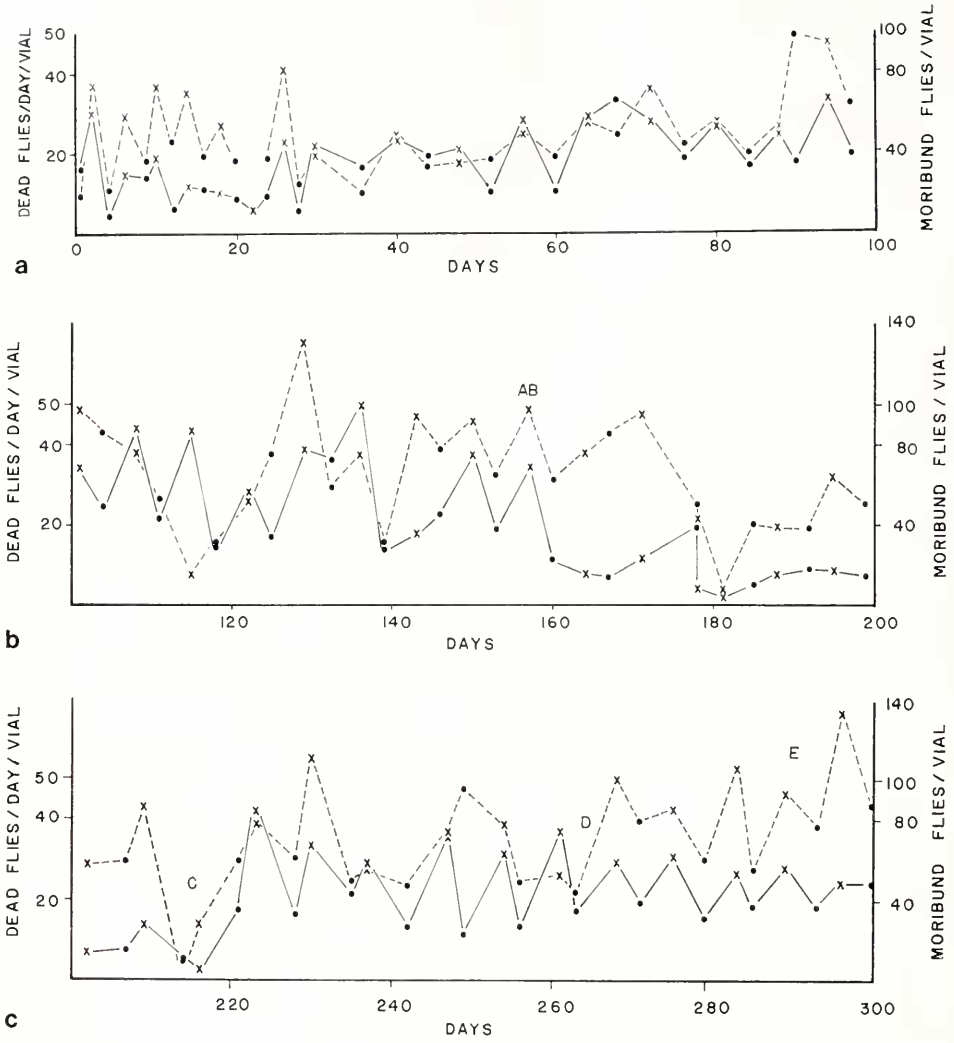
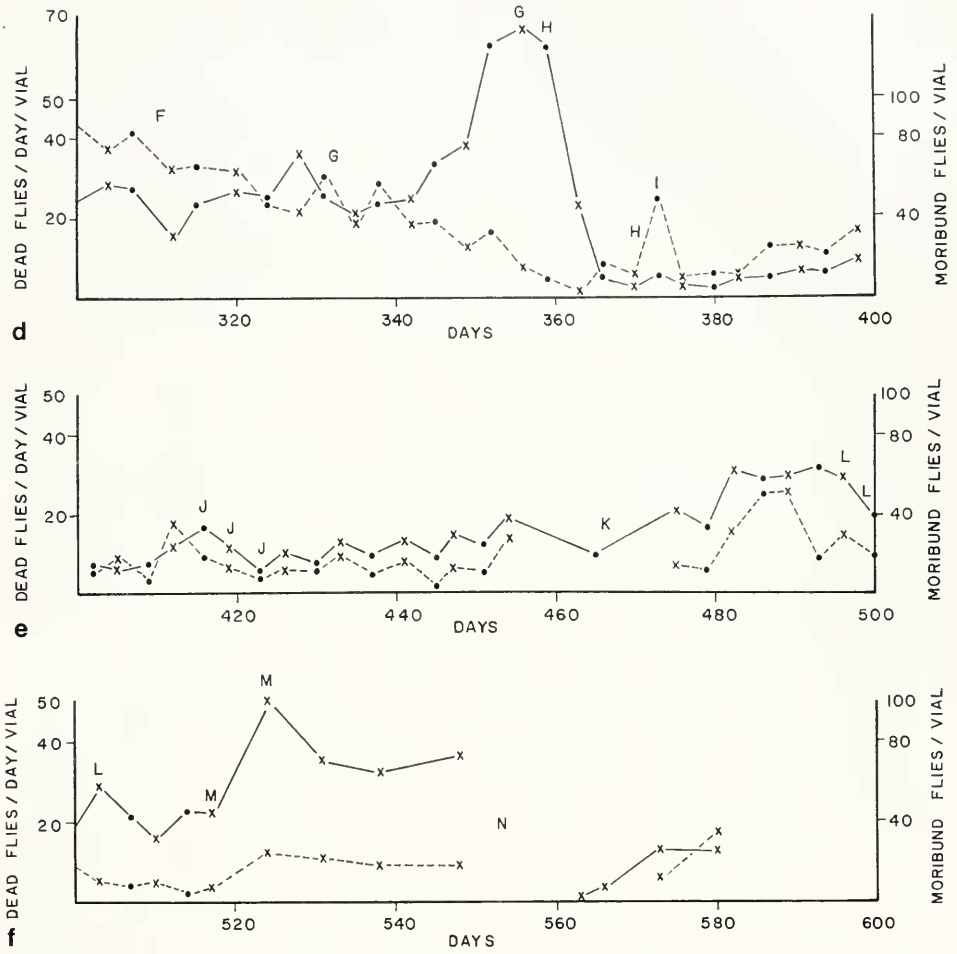


FIGURE 1. Numbers of dead flies per vial per day (continuous line) and number of flies per vial (dashed line) in right (cross) and left (filled circle) death vials. For Day 0–156, samples were averaged from 9 cages. At A (Day 157), samples were averaged from 4 cages from this date on. At B (Day 157), cages were turned around, so that death vials were nearest the wall. At C (Day 216), cages were turned back to original positions. At D (Day 264), death vials were covered with aluminum foil. At E (Day 290), the right side of each cage (side nearer the light) was covered with aluminum foil. At F (Day 310), the entire cage and food vials were shielded by loose fitting foil or mylar hood. Through G (Days 331 through 356), no new food vials were added in this period; no culture vials were removed. At each H (Days 359, 363, 366, 370), 4 fresh food vials were added to each cage on each of these days. At I (Day 373), all aluminum foil coverings were removed from cages. On J (Days 416, 419), short (45 mm deep) death vials replaced the regular vials removed for counting; the first counts from short vials were made on day 423. At K



(Day 466), all short death vials were replaced by regular (95 mm deep) vials. On L (Days 496, 500), short death vials replaced the regular death vials removed for counting; the first counts from short vials were made on day 503. At M (Day 517), both short death vials were removed. A single regular death vial was placed in each cage; the first counts from the single vials were made on day 524. At N (Day 553), all vials from each cage were cleared of flies; each set was placed in a clean, empty cage. Between days 380 and 400 substantial samples were taken from the cages for purposes of genotyping. The low values subsequently seen can be attributed to the reduced population size (in addition to the addition of a large number of fresh food vials).

progeny were left by the transferred moribund flies, and subsequent specific tests of fertility showed that essentially all moribund flies of both sexes were fertile when transferred to uncrowded conditions.

The absence of any indication of sharp heterogeneity among the moribund flies is important. While a two-step death curve would have indicated a mixture

TABLE II

Estimates of length of moribund period

Sample period	Moribund Flies per vial	Death Rate per vial	M/d**	
			Days	Hours
Standard I	65.67	36.10	1.82	44
Reverse	51.88	15.85	3.27	78
Standard II	65.60	34.54	1.90	46
Foil and Hood	74.82	34.27	2.18	52
Average, Rows 1, 3, and 4	67.44	35.52*	1.90	46

* Or 71.04 per cage.
** Number of moribund flies divided by death rate.

of dying and normal (presumably transient) flies, or else a two-step degenerative process, the apparent homogeneity of the moribund flies permits them to be treated tentatively as a single class. Thus the apparent 2-day duration of the moribund period must be taken as representative of the experience of each fly. Accordingly, the moribund flies, by leaving the death vials on occasion, may be capable of responding to altered conditions in the cage. Perhaps they can remain in the cage proper if its density has dropped, or if the competition has lightened.

A test of this possibility was performed by exchanging the death vials of a wild-type cage and one containing a population of *vestigial sepia* (*vg se*) flies. The latter strain is unable to compete with wild flies except under unusual circumstances. When the *vg se* death vials were placed in the wild cage, no flies left them to enter the cage. It should be noted that flies leave vials primarily on foot, so the absence of functional wings is not in itself a big drawback to the *vg se* flies. In the reciprocal maneuver, nothing happened at once, but eventually some moribund wild flies did leave their death vials and enter the *vg se* cage proper. Their movements were slow and halting. Eventually a few reached food. Some time after, and looking more robust (moving faster and appearing less shriveled), they seemed to take up positions and to threaten approaching *vg se* flies. These observations are anecdotal and must be understood in this spirit; for what they are worth, the wild flies executed sharp wing movements at the approach of *vg se* flies, and the latter turned away. Furthermore, a large number of *vg se* flies were soon in the death vials. Systematic observations of such encounters obviously constitute an important next step in the investigation of this whole subject. Eighteen days after the transfer, large numbers of wild flies began to appear in the cage, obviously progeny of the moribund wild flies that had left the death vials.

It should be noted that the criterion of moribundity depends in part on the intensity and persistence with which death vials are tapped and rattled before being removed. While light tapping results in the prompt departure of some flies, continued and heavier rattling causes more flies to leave. After a recent decision to increase the severity of the rattling, the numbers and proportions of flies counted as moribund have dropped. Estimates of durations of the moribund period, each averaged over several weeks' observations, are given in Table III.

TABLE III

Estimates of length of moribund period, considering only flies remaining after prolonged and intensive rattling

Cages	Conditions	M/d* (hours)
1,3,5,7	Standard	24.8
1,3,5,7	Vials in mid-cage position	42.5
2,4,6,8	Standard	23.7
2,4,6,8	Short vials	8.0
2,4,6,8	One regular vial per cage	12.2

* Number of moribund flies divided by death rate.

Another experiment employed genetically marked flies to get an idea of the age-distribution of the dying flies. In each of two cages, six vials of *net black cinnabar brown* (*n b cn bw*) flies were inserted for a period of three days, replacing regular culture vials. These *n b cn bw* cultures were just ready to produce their first adults; the strain is quite robust and competes well with wild flies in the laboratory. Eventually, a total of 167 *n b cn bw* flies appeared in the death vials, and of these 64 were found in the first three days. The rest appeared sporadically in declining numbers until 40 days later, after which none appeared for a considerable period. Meanwhile 72 had been found in the cage food vials that were replaced on a regular schedule. Since the *n b cn bw* cultures contained no adults when placed in the cage, 64 of the 239 that hatched during the three-day period entered the death vials at an early age: three days at most, and many must have been much younger. A few looked quite callow when found. Thus, the death vials receive adult flies of all ages, and if it is the weaker flies that become moribund, age is not the only cause of the weakness. Both these cages, a year later, contained numerous flies showing one or more of the recessive mutant phenotypes. (Other cages have been maintained with polymorphisms at these loci—and fairly even allele frequencies—for three years in our laboratory). Thus the appearance of large numbers of young *n b cn bw* flies in the death vials probably reflects the events characteristic of normal populations rather than the quick demise of an inferior stock.

In order to estimate the rate of emergence of adult flies (and therefore the death rate, given a steady state), half of the producing culture vials were removed from each of two cages. Emergent flies were counted and removed daily for three days, leading to an estimate of emergence per day of 86 for one cage and 198 for the other. The flies in each cage were counted directly *in situ* (this is tedious but practical). The first cage contained about 1570 flies; the other, 4010. Mean adult longevity thus is computed to be $1570/86 = 18$ days for the first cage and $4010/198 = 20$ days for the second. The remaining 9 cages, similarly counted, averaged 2468 flies (range = 1850–3355). With a mean adult longevity of 19 days, a mean daily emergence (and death) rate of 130 is indicated. Over long periods of time (Table II), the death rate for the two death vials of a cage, taken together, averages over 71. In computing the death rate, the moribund flies must

of course be included. The total number of dead and moribund flies is divided by the duration of the vial's stay in the cage and by the number of replicate vials. The mean death rate of 71 flies per cage per day includes all periods except the "reversed" period.

When cages contain no empty vials, the newest food vials often contain very large numbers of males and females. Many of these are dead and dying, so that in ordinary cages each food vial serves as a graveyard (as well as a nursery simultaneously) in its turn. This dual role is illustrated by the following observation. When one rattles a death vial repeatedly, some flies leave, but many remain. These are of course the moribund flies. When one rattles a crowded newly-placed food vial in the same cage, essentially all the flies leave. The critical test is now made on newly-placed food vials in cages without death vials: many flies remain in the vial. As in the death vials, they may climb half way up the vial, but they keep dropping back. These observations were made 3–20 hours after emplacement of the vials. Clearly, the immediate distinction resides in the flies and not in the physical differences between empty vials and those containing food. In this context it may also be noted that short vials (45 mm deep) seem to serve equally well as death vials. Moreover, recent experiments show that a *single* death vial accumulates over half the flies that die in the cage. Regular-sized (25×95 mm) vials were used in 4 cages, whose adult emergence rate was later measured by removal of all producing vials and the counting of all emergent flies each day for two days; then all the vials from each cage were placed in a clean cage, whose population was then counted directly after several days. After the vials had been removed from the old cages, the flies in each cage were removed, sexed, and counted. From these counts, and from adult emergence rates, adult longevities were estimated for each sex, as shown in Table IV. These

TABLE IV

Mean adult longevity estimates for four 1½-year-old cage populations

Sex	Mean adult emergence per cage per day	Mean cage population	Mean adult longevity, days
F	37.4*	1133.0**	30.3
M	33.5	1419.5**	42.4

* Proportion of females, 0.527; proportion in three other cages, 0.536.

** Some flies of unknown sex were added in the proportions observed; they constituted well below 5% of the entire sample.

estimates are considerably higher than those made over a year earlier when the cage populations were newer. The average death vial rate was about 41 at this time.

While the numbers are dramatically lower in the new food vials of cages containing empty vials, it is likely that some flies do die preferentially here as well. Furthermore, under conditions where the medium pulls away from the glass, many flies are trapped in the resultant crevices. The early estimates of death rates (130 overall and 71 in the death vials) differ by about 60. Over a year

later the respective estimates of 71 and 41 are lower but in a comparable ratio. Those flies that do not die in the death vials presumably find similar, though ostensibly less specific, conditions, such as the new food vials may offer. And others may die in random places. The proportions of these respective groups are not known.

When death vials are removed for counting, they are plugged with cotton and left for up to an hour before the counts are made. At the end of this time, some of the living flies look anything but moribund: they are active and well coordinated in every obvious respect. Indeed, some flies begin to look normal a few minutes after removal of the death vial. This observation indicates the possibility of a depressing olfactory—or even auditory—stimulus from the cage, a stimulus whose removal is accompanied by a recovery of vitality by some of the moribund flies.

We have tried without success to analyze emigration in sets of vials connected by tubes of various sizes. A variety of mixed populations were used. From these experiments and from the observation of cages, where most of the healthy flies are not in the culture vials, we have concluded that an element is missing from such theaters, and that is abundant flat surface. Accordingly, our further experiments are being done in standard and special cages. In the course of these experiments, a distinct additional form of behavior has been noted and studied. It will be described in a subsequent publication.

Discussion

Clearly, the flies accumulate in a specific place in the cages before dying. What do we know about the process, and what questions remain?

First of all, the death vials are not merely traps. Some flies can easily leave the vials, and healthy flies placed in empty vials have no trouble escaping. As noted, shorter vials serve well as death vials. Also, if we take the estimates of the duration of the moribund period seriously, moribund flies do leave the death vials at least once or twice on the average to get food and water. To recapitulate the reasoning, moribund flies placed in closed food vials survive well and behave as a unimodal class; they live only half as long as ordinary healthy flies under these conditions. Healthy flies live only about a day without food, the moribund flies appear to live about two days in the death vials. Thus they must be going out for sustenance once in a while. The decisive observation has not been made, however: moribund flies must be seen to leave the death vials, enter food vials, and return. Obviously, too, there must be a progressive decline of vitality toward death. Presumably, the flies that do not leave the death vials even after extreme disturbance are in a weaker state than those that do. Nevertheless, the state of moribundity holds interest in its initial as well as more extreme stages.

The behavior of the moribund flies suggests impending death. Their movements are erratic, uncoordinated, often non-adaptive, and quite reminiscent of the familiar behavior of moribund houseflies. But what organized activity brings them to this particular spot? Where have the flies been just before entering the death vials? What interactions, if any, with other flies preceded their departure? Is the departure a form of emigration, a form of suicide, a set of both, or neither?

It seems to me that the flies are emigrating. The unusual circumstances make them emigrate into captivity, but that may well be a frequent occurrence in nature, too. The time comes when each fly has to take his chances elsewhere. And perhaps this time is announced to the fly in a series of interactions whose model is seen in territorial behavior.

Naturally these observations will call to mind what Wynne-Edwards (1962) has said so well. Perhaps the continuous nature of the emigration is novel, however. Wynne-Edwards emphasized the "safety-valve" aspect of emigration and discussed it solely as an intermittent (regular or irregular, but not continuous) activity in populations. In other discussions (Dingle, 1972; Johnson, 1969; Krebs, Gaines, Keller, Myers, and Tamarin, 1973) as well, the sporadic or cyclical aspects of emigration are emphasized. The *Drosophila* cages, in contrast, present an example of continuous, though modulated, emigration. When the endpoint of migration is an empty vial, socially-induced emigration and socially-induced mortality are one and the same thing.

There is nothing new about the departure of queen bees from a hive; the exclusion of young gulls or young male blackbirds from specific territories; or the flight of refugees before human conquerors. In general, however, we think of territories as more or less fixed. The stickleback knows his borders; so do many birds. Nevertheless, there is nothing essential to the fixity of territory. Granted, permanent territories add stability to a colony, and they are easy to observe. But a given area can be subdivided among a number of moving individuals, each of whom may during a stationary period or even while in motion drive away others who come too close. If this does not fit the conventional concept of territoriality, then it is certainly prototerritoriality. In any event, I believe such behavior may underlie the accumulation of the flies in the death vials.

When a fly approaches a stationary male, the latter flutters its wings, and the former generally moves on. Often one male will pursue another, poking at its abdomen as in courtship. These interactions are the type that could add up to a departure to the death vial, but of course we have no explicit information on the question. But whatever the specific interactions, the model of an area dynamically saturated with floating territories (or "personal space") may serve as a guide to the design of further experiments. While such a model does not answer a lot of specific questions (*e.g.*, how does a fly know when to go to the death vial?), it makes them quite analogous to questions for which we have straightforward answers (a herring gull flees from a hostile opponent whose threat behavior indicates superior strength and a determination to attack). The critical observation here is the path of a fly to the death vial. No doubt a movie played backwards could identify the beginning.

Aggressive behavior and non-random spacing have been noted in experimental populations of *Drosophila* from time to time. While Hay (1973) did not discriminate among a variety of activities in *D. melanogaster*, he was aware that some of them were likely to fend off other flies. Manning's (1959) description of wing-flicking fits what I have referred to earlier; Connolly (1968) recognized uniform spacing as possibly significant and attributed an increase in "preening" (activity without translocation) to the visual perception of other flies. Sexton and Stalker (1961) noted that the upper surface of a *cylindrical* chamber was choice.

In their experiments, at most 183 flies occupied the ceiling, even with as many as 1,000 flies in the chamber. Under crowded conditions, they report intriguingly, some flies were on the wall and many were piled up on the floor. The extension of legs seemed to ward off other flies; this behavior was apparent when another fly came within about 5 mm of a stationary individual. While no specific (permanent) territory was defended, Sexton and Stalker (1961) noted that the uniform spacing might nevertheless "reduce competition." Naylor (1959) showed clearly that *Tribolium confusum* (flour beetle) populations have a density-dependent spacing pattern, with crowding resulting in spacing more uniform than a random distribution. Clark and Evans (1954) treat the analysis of spacing in a clear and thorough way. These several observations and interpretations of spacing patterns can usefully be thought of together with the phenomenon of emigration, which Lidicker (1962), among others, has considered as a possible component of a mechanism regulating population density. The experiments of Sakai, Narise, Haraizumi, and Iyama (1958) on emigration in *Drosophila* deal with unit time periods considerably longer than those employed here; more important, perhaps, discrete culture generations were employed, and they used large vials connected by tubes. Taken together, the observations cited indicate the common use of behavior resulting in even spacing, the possible coupling of spacing to emigration, and the occurrence of emigration on a scale sufficient to have a major impact on population density.

The importance of this behavior lies in its role in the control of population density. Mass-action-limits debilitate the population, and in the absence of grazing and similar causes of death, mechanisms must be found to prevent all the individuals from living on the brink of starvation. In fact, cage flies are invariably a lot larger than the tiny but fertile individuals one can produce experimentally by a judicious manipulation of the medium. As Wynne-Edwards (1962, p. 10) says, ". . . the ceiling is normally imposed, and the level indefinitely maintained, while the members of the population are in good health—sometimes actually fat—and leading normal lives." There are two evident behavior patterns in *Drosophila* cages that make for a less-than-maximal partitioning of the environment. First, many larvae leave the food prematurely under crowded conditions. Circumstances arise which cause them to take their chances elsewhere; in a cage, they starve on the sides of the vials. This leaves enough food (and perhaps other resources) for those who remain to metamorphose into robust adults. And secondly, adult flies go to the death vials before it is physiologically necessary to die. In each case, the individuals die on cue. Cues, weak signals amplified by the receiver, are essential to regulation.

Why should an individual face certain death rather than settle for a meager existence? The answer, again, lies in territoriality. Even where death by attack is not possible, evolution can program individuals to prefer a step towards death to receiving a certain stimulus, to choose isolation over insult, to find challenge more agonizing than deprivation. The context of this preference may lie in the same appreciation of superior competition that turns many a bird, insect, and mammal away from a displaying rival. Then what of the *insensitive* inferior individual? He may survive longer, but he will not prosper. The cost to the population will not be great, and there is nothing to make the property of insensi-

tivity spread. Also, it may be that no single behavioral mutation can be so deft as to leave an individual immune to the signal to go away, yet perfectly capable of using all other information from his environment.

Most individual organisms do not live a continuously marginal existence. Resources are usually partitioned so that each share is well above the minimal subsistence level. While many natural populations may be kept below deleterious densities by outside forces, and while others may oscillate and occasionally be cut back by actually reaching such densities, a *Drosophila* cage population's density is limited by factors intrinsic to the population itself. Individual larvae and individual adults must be able to choose a new quasi-developmental track (towards moribundity) in response to environmental cues, in the same sense that crowded locusts choose to emigrate, certain termites choose to become soldiers, and tissues in a broken salmander limb choose to regenerate. At a fork in the road, one direction is taken; the other is not. This is not rational choice, but it is a choice.

Presumably a cage can be made to have a density sufficiently low to eliminate death on cue. At such a density, perhaps the death vials would receive only their random share of moribund flies. Alternatively, perhaps *all* moribund flies are inclined to seek out such death sites, whether or not they die before the final possible time. In any event, the nature of the cues and of the flies' initial responses to them should be of great intrinsic and general significance.

In various sorts of population cage studies, the ability to monitor death will have useful applications. For example, a comparison of genotype frequencies between newly-emerged and newly-dead flies will indicate whether the population is in a steady state. If it is, comparison of genotype frequencies between newly-dead flies and the general population can indicate whether certain genotypes influence longevity and therefore, presumably, fitness. Indeed, longevity may be a critical variable in the operation of selection—and perhaps the maintenance of genic polymorphism—in cage- and natural-populations.

Of course the death-vial death rate is apparently just over half the overall death rate, and it may well be a variable fraction, at that; but if conditions can be devised which increase and stabilize this fraction, then the resultant ease of obtaining death-rate estimates will be quite valuable. It is obviously far easier and less disruptive to monitor death vials than to monitor producing culture vials. Since one or the other must be done to estimate generation time (and in turn track progress toward linkage equilibrium, for example), the development of conditions favoring the death vials as the place of death is most desirable.

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Summary

More than half the flies that die in a *Drosophila melanogaster* population cage do so in empty vials if they are provided. Before dying, the flies exhibit characteristic erratic behavior; if placed in uncrowded conditions they are fertile and

they live for several weeks. This phenomenon is neither light-dependent nor exclusively age-dependent. Crowding is clearly important.

It appears that the healthier flies maintain moving territories, keeping others at a distance and thus minimizing crowding. The others emigrate, in this case into a resourceless chamber, so that socially-induced emigration becomes socially-induced death. This physiologically unnecessary death is viewed as a component of an intrinsic population-density-regulating mechanism in *Drosophila melanogaster,* and presumably in many other organisms that have no fixed territories.

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